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BAYER AG

Document Title

SALMONELLA/MICROSOME TEST WITH DESMODUR T 100 WITH COVER LETTER DATED 051193

Chemical Category

TOLUENE-2,4-DIISOCYANATE (584-84-9)

Section 8d

MILES

Miles Inc
Mobay Road
Pittsburgh, PA 15205-9741
Phone 412 777-2000

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May 11, 1993

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Attention: 8(d) Health and Safety Reporting Rule
(Notification/Reporting)

Gentlemen:

Enclosed is a copy of a Health and Safety Study that was just received from our parent company Bayer AG. We are submitting this study on behalf of Miles Inc., Mobay Road, Pittsburgh, Pennsylvania 15205. We are filing this Health and Safety Study to comply with the regulations codified at 40 CFR, Part 716. This submission contains no Confidential Business Information (CBI).

The information required at 40 CFR 716.30 is given below.

Chemical Name:	Toluene-2,4-diisocyanate
CAS No:	584-84-9
Name of Study:	Salmonella/Microsome Test: Study # T5039111
Submitting Official:	Francis J. Rattay
Title:	Manager, Regulatory Affairs
Address:	Mobay Road Pittsburgh, Pa 15205
Telephone No.:	(412) 777-7471

8693000217

Sincerely,

Francis J. Rattay

Francis J. Rattay
Manager, Regulatory Affairs
(412) 777-7471

Attachment
Certified Mail No.: P 213 126 281

86930000217

B A Y E R A G
FACHBEREICH TOXICOLOGY
Friedrich-Ebert-Straße 217-333
D-5600 Wuppertal 1, F.R.G.

Report No. : 22167
Report Date: 1.4.1993

Desmodur T 100

SALMONELLA/MICROSOME TEST

Study No.: T 5039111

by

Dr. R. Gahlmann

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GLP Certification by Study Director

Compound : Desmodur T 100
Study No. : T 5039111

This study conforms to OECD Principles of Good Laboratory Practice (Bundesanzeiger Nr. 42a of the 2nd of March 1983 and Bundesgesetzblatt, Part I, of the 22nd of March 1990).

R. Gahlmann

Dr. R. Gahlmann

Wuppertal, November 23, 1992

CONTENTS

Declaration by Quality Assurance Unit

Compound : Desmodur T 100
Study No. : T 5039111

The laboratory in which this study was performed has been inspected by Quality Assurance on the dates indicated below. The results of the checks and inspections are conveyed in writing to the study director and, if necessary, also to the Head and Director of the Institute, or other responsible persons.

Date of check/inspection	Date of issue of inspection report
Mar. 24, 1992 (study plan)	Mar. 24, 1992
Sep. 16, 1992	Sep. 16, 1992

The results of this study and the methods used have been correctly reported.

Quality Assurance Unit
PH-AQ-S/GLP, Bayer AG

Date: March 25, 1993 Responsible: Dr. H. Lehn

1. Signatures

Study Director : R. Gahlmann — Dr. R. Gahlmann — MAR 29, 1993 — Date

Section Head : Dr. B. Herbold — MAR 29, 1993 — Date

Head of Institute: Dr. E. Löser — 30. März 1993 — Date

BAYER AG

2. Summary

The mutagenic potential of Desmodur T 100 was examined in the Salmonella/microsome test. Bacteria of four histidine-auxotrophic Salmonella typhimurium LT2 mutant strains (TA 98, TA 100, TA 1535, and TA 1537) were exposed to doses up to 5000 µg per plate.

Doses up to and including 125 µg per plate did not cause any bacteriotoxic effects: Total bacteria counts remained unchanged and no growth inhibition was observed. The substance revealed weak, strain-specific bacteriotoxic effects at higher doses up to 1000 µg per plate. Strong bacteriotoxic effects were observed for the doses of 2000 µg, 4000 µg and 5000 µg per plate. Substance precipitation occurred at the dose of 800 µg per plate and above. Plates corresponding to the dose of 2000 µg per plate and to higher doses could not be assessed.

There was evidence for mutagenic effects of Desmodur T 100 with S9 mix. A biologically relevant increase of the mutant count over control levels was observed at the dose of 100 µg per plate and at higher doses with Salmonella typhimurium strains TA 98 and TA 1537. Therefore, Desmodur T 100 was considered to be mutagenic with S9 mix in the Salmonella/microsome test. A positive response was found only with S9 mix. The lowest reproducibly effective dose was 200 µg per plate for Salmonella typhimurium TA 98 and 400 µg per plate for TA 1537. The Salmonella/microsome test thus showed Desmodur T 100 to have a weak but definite mutagenic effect under the test conditions.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene revealed marked mutagenic effects, as indicated by a biologically relevant increase of mutant colony numbers over colony numbers of the negative controls.

3. Introduction

The mutagenicity evaluation was performed by using the Salmonella/microsome test (Ames Test) as described by Ames et al. (1973a, 1975) and Maron and Ames (1983).

The Salmonella/microsome test is a screening method which allows to assess whether point mutations are induced by chemicals in the genome of Salmonella typhimurium test strains in vitro. Bacteria of auxotrophic mutant strains are exposed to the chemical agent and the number of revertants to a prototrophic phenotype is compared to the number of spontaneous revertants. A test agent is considered to be mutagenic if the rate of reversion increases significantly and reproducibly after treatment.

The mammalian metabolism which is an important factor in chemical mutagenesis is simulated in this test by the 9000 g fraction of homogenized mammalian livers. S9 mix which is composed of this liver cell extract, supplemented with cofactors, is added to the test system in order to mimic the metabolic features of mammalian cells.

The method itself is considered to be very sensitive (Herbold et al., 1976; Herbold, 1978) and is well suited for fast screening. Available literature indicates a high correlation between the positive and negative responses of the Ames assay and the carcinogenic activity of the tested substances (McCann et al., 1975a, 1976; Purchase et al., 1976, 1978). In addition, the test represents a good screening system for potential carcinogenic effects, although the results should not be overrated, as this high correlation may not apply to all substance groups (Ames, 1979; Andrews et al., 1978; Clayson, 1980; Glatt et al., 1979 and Rinkus and Legator, 1979; Zeiger, 1987).

The test was performed at the Institute of Toxicology for Industrial Chemicals, Fachbereich Toxicology, BAYER AG, Friedrich-Ebert-Straße 217-333, D-5600 Wuppertal 1, F.R.G.

Study initiation date:	March 12, 1992
Study start date:	Sept. 16, 1992
Study termination date:	Nov. 9, 1992
Study completion date:	report date (see front page)

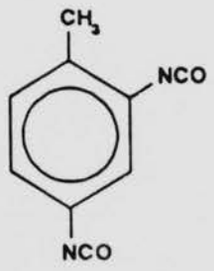
The records are filed in the Fachbereich's archive.

4. Material and Methods

4.1 Substances

4.1.1 Test Substance

name of test substance	:	Desmodur T 100
order number	:	BALK 92/021
manufacturer	:	BAYER AG
product number	:	409 383-00
sample number	:	390052
content	:	99.8% (analytical result dated July 16, 1992)
approved	:	until February 16 ,1993
appearance	:	colourless, clear liquid
storage	:	at room temperature
chemical name	:	Toluene-2,4-diisocyanate
structure	:	



molecular weight	:	174.2 g/mole
molecular formula	:	$C_9H_6N_2O_2$
CAS No.	:	584-84-9

intended use : industrial chemical

The batch used was analysed prior to study initiation and approved for use during the test period. A stability test did not reveal any significant change of the concentration of the active ingredient in the solvent over the test period.

4.1.2 Positive Controls

Sodium azide (Na-azid, SERVA), order no. 30175 (Control:D), a direct-acting mutagen used as specific positive control for TA 1535.

Nitrofurantoin (NF, SERVA), order no. 30600 (Control:A), a direct-acting mutagen used as specific positive control for TA 100.

4-nitro-1,2-phenylene diamine (4-NPDA, Merck), batch no. VV452057, a direct-acting mutagen used as specific positive control for TA 1537 and TA 98.

2-aminoanthracene (2-AA, EGA-Chemie), batch no. 7413406, a promutagen which reverts all the strains and serves as a control for the activating effect of the S9 mix.

The positive controls sodium azide, nitrofurantoin and 4-nitro-1,2-phenylene diamine were only used without S9 mix; the positive control 2-aminoanthracene was only used with S9 mix.

4.2 Indicator Organisms

4.2.1 Description of Test Strains

Histidine-deficient mutant strains of Salmonella typhimurium LT2 served as indicators of point mutagenic effects. The strains were selected specifically for the Salmonella/microsome test. Point mutations can be divided into two basic classes, base-pair substitutions and frameshift mutations. Thus, strains that allow to assay for both classes of mutations were included in the collection of test strains.

They included the Salmonella typhimurium strains TA 1535 and TA 1537 selected by Ames et al. (1973b) and TA 100 and TA 98 developed by McCann et al. (1975b). Both strain TA 1535 and TA 100 bear the base-pair substitution his G 46. TA 100 carries the plasmid pKM 101 which encodes the muc+ gene that increases the resistance to the lethal effects of many mutagens at the expense of increased mutability and an ampicillin resistance gene as a selectable marker. The same R factor is also present in strain TA 98. TA 1537 and TA 98 bear the frameshift markers his C 3076 (a +1 mutant) and his D 3052 (a +2 mutant) respectively.

All strains have two additional properties in common which increase their sensitivity. Firstly, they are deep rough since certain lipopolysaccharide side chains are missing in the bacterial cell wall. Larger, mutagenic molecules can therefore enter the cell and cause mutations. Secondly, their reduced ability to repair damage from UV light (e.g. thymidine dimers) allows the phenotypic detection of mutation events which would otherwise remain undetected.

Strain TA 1535 is commonly used in addition to strain TA 100, while TA 1538 is normally not used in addition to TA 98. This has two reasons:

A) There is no relevant increase in the spontaneous mutant counts of TA 98, compared to the spontaneous range of TA 1538. Special differences in sensitivity existing between TA 1535 and TA 100, which are attributed to the relatively high spontaneous rate of TA 100 (10 times that of TA 1535), do not exist between TA 1538 and TA 98. b) An international general inquiry has shown, that using TA 1538 in addition to any of the test strains in this study would not provide further information of biological relevance (Herbold, 1983).

This is in agreement with international guidelines, as published by the OECD, EEC, or EPA. Strain TA 1538 was either deleted in these guidelines, or never introduced at all. Maron and Ames (1983) also reported: "Although TA 1538 is useful for the detection of particular aromatic frameshift mutagens such as 4-nitro-o-phenylene diamine, we decided to drop the strain because it overlaps considerably with TA 98.

TA 1538, which differs from TA 98 in lacking the plasmid pKM 101, is used in spite of these considerations, if questionable TA 98-results need clarification. This was not the case in the present investigation.

4.2.2 Origin of Strains

The original strains were obtained from Prof. Bruce Ames and arrived at the Fachbereich Toxicology, BAYER AG, on December 12, 1986.

4.2.3 Production of Stock Cultures

The samples were inoculated immediately upon receipt onto nutrient agar plates and incubated at 37°C for approximately 24 hours. Plates and medium that were used for the cultivation of strain TA 100 and TA 98 at this step and during following selection procedures contained ampicillin. Nutrient broth was inoculated with single colonies and cultures for each strain were grown over night at 37°C. Bacteria from each culture were then grown on nutrient agar plates.

After an incubation period of approximately 24 hours at 37°C, new samples of individual colonies from these plates were transferred to flasks containing approximately 30 ml of standard nutrient broth. The culture was incubated overnight at 37°C. Thereafter, a small sample was removed to check the genotype. The remaining cultures were treated with DMSO to protect against the effects of freezing, and immediately frozen in portions of 1 ml at -80°C (Ames et al., 1973b; McMann et al., 1975b). No additional ampicillin-resistance tests were required for strains TA 98 and TA 100 since the bacteria had already been grown under ampicillin selection.

The crystal-violet sensitivity test (to confirm the deep rough phenotype) and UV sensitivity test (to confirm the phenotype) were performed as described below. Frozen cultures which did not meet the criteria were discarded. Remaining cultures were stored for future testing. Frozen cultures of batches that produced results deviating from expected values for negative or positive controls during mutagenicity testing were also discarded.

Whenever new stock cultures were needed, cultures were inoculated from single colonies from stock plates which contained ampicillin in the nutrient agar for the strains TA 100 and TA 98. The cultures containing approximately 30 ml of nutrient broth were incubated, stored in aliquots of 1 ml, and checked for crystal-violet and UV sensitivity.

One 1 ml-portion was thawed for each test and strain, and quantities of 0.2 ml of the thawed culture were added to 10 ml nutrient broth. This culture was incubated overnight at 37°C and used only on the same day. Thus, each test was performed with bacteria that had been grown from aliquots of a small stock culture whose properties had been checked immediately before freezing. In general, this obviated any need to re-check the genotype for each Salmonella/microsome test. This procedure is in accordance with the methods described by Ames et al. (1975) and Maron and Ames (1983).

4.2.4 Checking of Genotype

4.2.4.1 Histidine Requirement

In each individual test, histidine dependence of the cultures was automatically checked by the accompanying negative controls. The number of mutants of each individual plate is listed in the Tables 1 to 20.

4.2.4.2 Ampicillin Resistance (pKM 101)

A special test for ampicillin resistance was not necessary since strains TA 100 and TA 98 were incubated on ampicillin containing nutrient agar. Consequently surviving bacteria were ampicillin resistant.

4.2.4.3 Crystal-Violet Sensitivity (deep rough)

A volume of 0.1 ml was taken from individual stock samples and spread on nutrient agar plates (four plates per strain). After a few minutes, filter papers to which 10 µl of an aqueous crystal-violet solution (1 mg/ml) had been added were placed in the middle of the plates. The plates were incubated overnight at 37°C and the diameters of the inhibition zones that had formed were measured. The inhibition zones of all batches of stocks that were used for mutagenicity testing revealed adequate sensitivity to crystal-violet.

4.2.4.4 UV Sensitivity (uvrB)

Samples were spread onto nutrient agar plates as described under 4.2.4.3. One half of each plate was covered with aluminium foil and irradiated with UV light of a wavelength of 254 nm at a distance of 33 cm without a lid for six seconds (TA 1535 and TA 1537) or eight seconds (TA 100 and TA 98), respectively. The irradiated plates were incubated as described under 4.2.4.3 and inspected. Adequate sensitivity was demonstrated if no bacteria had grown on the irradiated half of the plate. This was the case with all batches of stocks that were used for mutagenicity testing.

4.2.5 Stock Batches

Stock Batches Used in Tables				Strain
1-4	5-12	13-16	17-20	
20.03.92/2	24.07.92/2			TA 1535
24.07.92/1	24.07.92/1			TA 100
20.03.92/1	20.03.92/1	24.07.92/1	24.07.92/2	TA 1537
24.07.92/1	24.07.92/1	24.07.92/1	17.09.92/2	TA 98

4.3 S9 Mix

S9 mix was used to simulate the mammalian metabolism of the test substance. It was prepared from the livers of at least six adult male Sprague Dawley rats of approximately 200 to 300 g in weight. For enzyme induction, the animals received a single intraperitoneal injection of Aroclor 1254, dissolved in corn oil, at a dose of 500 mg/kg body weight, five days before sacrifice. The animals were prepared unfasted, following the directions of Ames et al.(1975) and Maron and Ames (1983).

The rats were killed by cervical dislocation. Livers were removed under sterile conditions immediately after sacrifice and kept at 4°C until all animals had been prepared. All the remaining steps were carried out under sterile conditions at 4°C.

The livers were washed with a cold (+4°C) solution of 0.15 M KCl (approximately 1 ml KCl per gram of liver) and homogenized in fresh, cold (+4°C) 0.15 M KCl (approximately 3 ml KCl per gram of liver). The homogenate was then centrifuged in a precooled centrifuge at +4°C and 9000 g for 10 minutes. The supernatant (the S9 fraction) was stored at -80°C in small portions.

Aliquots of the frozen supernatant were thawed slowly before use. The S9 mix was prepared freshly each time (Ames et al., 1973a) and used only on the same day. Throughout the experiment, the mix was kept cold in a glass vessel with a double wall in which the space between the walls had been filled with ice water.

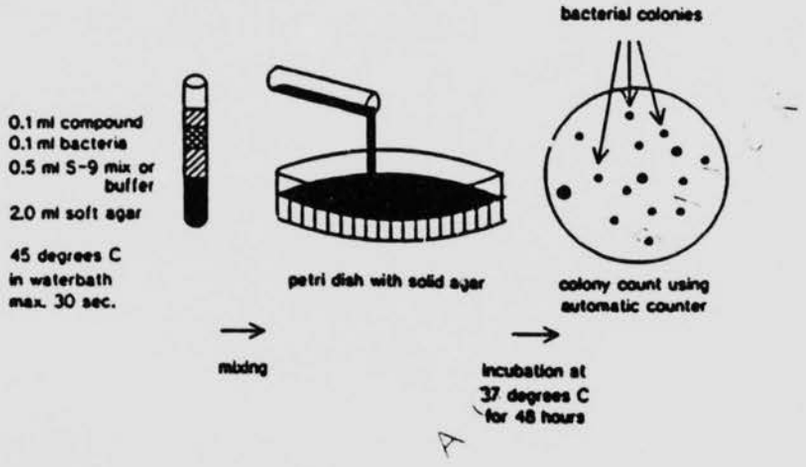
Seventy ml of cofactor solution contained:

$MgCl_2 \times 6 H_2O$	162.6 mg
KCl	246.0 mg
glucose-6-phosphate, disodium salt	179.1 mg
NADP, disodium salt	315.0 mg
phosphate buffer	100.0 mM

S9 mix consists of this cofactor solution, S9 fraction and, if needed, 0.15 M KCl. The amount of S9 fraction in S9 mix is indicated in Tables 1 to 20 in percent. The S9 mix comprised the amount of S9 fraction (x%) indicated in Tables 1 to 20, 70% cofactor solution and (30-x)% 0.15 M KCl. The S9 fractions were derived from the preparations dated January 20, 1992 and July 27, 1992 (protein content: 25.9 mg per ml and 27.2 mg per ml, respectively). Prior to first use, each batch was checked for its metabolizing capacity by using reference mutagen(s) and appropriate activity was demonstrated. At the beginning of each experiment 4 aliquots of the S9 mix were plated (0.5 ml/plate) in order to assess its sterility. This was repeated after finishing of test tube plating. The sterility control plates were then incubated for 48 hours at 37°C. No indication of contamination of S9 mix was found.

4.4 Test Protocol

The test followed the directions of Ames et al. (1973a, 1975) and Maron and Ames (1983).



Four tubes were plated per strain and dose for the mutant count, with and without S9 mix, respectively. As negative controls the same number of tubes with solvent minus the test substance was plated. Positive controls were also plated in quadruplicate. The amount of solvent that was used for the test substance and for the controls was 0.1 ml/plate.

In general, tubes were plated immediately after addition of the last component. In some cases, however, a preincubation of the test tubes was performed before plating. This was not the case in the present study.

The doses for the first trial were normally determined on the basis of a standard protocol: 5000 µg or 5 µl per plate were used as the highest dose unless the solubility was limiting. At least four additional doses were routinely used. If plates of fewer than three doses could be used for assessment purposes, at least two repeat tests were performed. The results of the first experiment were then regarded as a pre-test for toxicity. In case of a positive response, however, or if the plates of at least three doses could be used for assessment purposes, the first trial was included in the assessment. If the second test confirmed the results of the first test, no additional repeat test was performed. Doses of repeat tests were chosen on the basis of the results obtained in the first experiment.

The toxicity of the substance was assessed in three ways. First, background growth on the plates for mutant determination was inspected. If a reduction in background growth was observed, this indication for toxicity was indicated in the tables by the letter "b" after the mutant count. A single "B" without any numerical value for a mutant count represents four plates with reduced background growth at a given concentration. (The same applies to the symbols "C", "V", "P", "N" or "%", which may also appear in the tables.) Secondly, a toxic effect of the substance was assumed when the mutant count per plate was reduced significantly and in a dose-dependent manner as compared to the corresponding negative control. The third criterion was the bacteria titer. Total bacterial counts were taken on two plates with S9 mix for each concentration studied. If a test was performed only without S9 mix, however, the bacterial count was taken on plates without S9 mix.

The bacterial suspensions were obtained from 17-hour cultures in nutrient broth, which had been shaken at 37°C and at 90 rpm. Such suspension cultures were used for the plating experiments. No standardized procedure was employed to adjust the bacterial suspensions to a defined density of viable cells per milliliter, since the selected culture conditions normally produce cultures of the desired density. However, the numbers of viable cells in each culture were determined as part of the titration procedure. The numbers of viable cells are listed in Tables 1 to 20 as the negative control values.

The dilution of bacterial suspensions used for the determination of titers was 1:1,000,000. Plating for the titration and for mutagenicity testing was performed under the same conditions except that the histidine concentration in the soft agar was raised from 0.5 mM to 2.5 mM for the titration to permit unrestricted bacteria growth.

The tests were generally performed with and without S9 mix. Details of the results are compiled in Tables 1 to 20.

The plates were incubated at 37°C for 48 hours and bacteria colonies were generally counted immediately after incubation. If no immediate count was possible, plates were temporarily stored in a refrigerator.

The following criteria determined the acceptance of an assay:
a) The negative controls had to be within the expected range, as defined by published data (i.e. Maron and Ames, 1983) and our historical data (see Chapter 8).
b) The positive controls had to show sufficient effects, as defined by the laboratories' experience (see Chapter 8).
c) Titer determinations had to demonstrate sufficient bacterial density in the suspension.

Only assays which complied with all three of the above criteria were used for assessment. Furthermore, the data generated in this assay needed to be confirmed by two additional independent experiments. Even if the criteria for points (a), (b) and (c) were not met, an assay was accepted if it showed mutagenic activity of the test compound.

The following doses per plate were evaluated in the first test:

		µg per plate
1.	Negative control	0
2.	Desmodur T 100	5000
3.	Desmodur T 100	1000
4.	Desmodur T 100	200
5.	Desmodur T 100	40
6.	Desmodur T 100	8
7.	Positive control, sodium azide	10 (only TA 1535)
8.	Positive control, nitrofurantoin	0.2 (only TA 100)
9.	Positive control, 4-nitro-1,2-phenylene diamine	10 (only TA 1537)
10.	Positive control, 4-nitro-1,2-phenylene diamine	0.5 (only TA 98)
11.	Positive control, 2-aminoanthracene	3

Due to the substance's toxicity, doses ranging from 50 µg to 4000 µg per plate were chosen for the repeat tests. Individual doses are given in Tables 5 to 20.

The solvent employed for Desmodur T 100 was ethylene glycol dimethylether (EGDE) and for the positive controls DMSO.

The solvent for the test substance was selected based on information provided by the internal sponsor. The stability was determined in dry EGDE. The first experiments (Tables 1-16) were performed with regular EGDE. The results from these experiments were confirmed by tests (Tables 17-20) in which dried EGDE was used.

No "untreated" negative control was set up for EGDE since sufficient evidence was available in the literature (i.e. Maron and Ames, 1983) and from our own experience (see Chapter 8), indicating that this solvent had no influence on the numbers of spontaneous revertants with the bacterial strains used.

4.5 Assessment of Results

A test is defined as being positive if a reproducible and dose-related increase of mutant colony numbers becomes apparent for at least one strain. For TA 1535, TA 100 and TA 98 mutant colony numbers should increase by a factor of two or more over negative control numbers, while at least a three-fold increase should be apparent for TA 1537. Otherwise, the result is judged as negative. However, these guidelines may be overruled by good scientific judgement.

In case of questionable results, investigations should continue, possibly with modifications, until a final evaluation is possible.

4.6 Study Guidelines

The study was performed according at least to the following guidelines:

EEC Directive 84/449/EEC
B.14. Other Effects - Mutagenicity
Salmonella typhimurium
Reverse Mutation Test

OECD Guidelines for Testing of Chemicals
"Genetic Toxicology: Salmonella typhimurium,
Reverse Mutation Assay"
Adopted: 26 May 83, No. 471

New and Revised Health Effects Test Guidelines October 1984.
(U.S.) Environmental Protection Agency Washington, DC
(PB 84-233295).
HG - Gene Muta - S. typhimurium, October 1984

4.7 Study Identification and Responsibilities

4.7.1 Type of Test and Study Number

Salmonella/Microsome Test :T 5039111

4.7.2 Responsibilities

Head of Institute of Toxicology for Industrial Chemicals	:Dr. E. Löser
Section Head	:Dr. B. Herbold
Study Director	:Dr. R. Gahlmann
Senior Technician	:Mrs. M. Bönning
Head of Archives	:Dr. E. Löbbecke
Quality Assurance	:Dr. H. Lehn
Analysts	:Dr. Seelemann Hr. Malzer

5. Results

5.1 Description of Results

The mean values of the mutant counts for each set of plates are listed in Tables 1 to 20. There was no indication of a bacteriotoxic effect of Desmodur T 100 at doses of up to and including 125 µg per plate. The total bacteria counts consistently produced results in the range of the negative controls, or differed only insignificantly. No growth inhibition was observed. Higher doses revealed a weak, strain-specific bacteriotoxic effect up to the dose of 1000 µg per plate and the corresponding plates could be used for assessment purposes. The substance was highly bacteriotoxic at higher doses and plates could not be evaluated for assessment purposes.

The substance started to precipitate at the dose of 800 µg per plate. Plates corresponding to the dose of 2000 µg per plate or higher doses could not be evaluated.

The test strains TA 98 and TA 1537 revealed a dose-related, approximately two- to four-fold increase of revertant colony numbers over numbers for negative control plates. Reproducible increases were observed in these two strains in the dose range between 400 and 1000 µg per plate. Positive findings were obtained only with S9 mix.

Summary of the Results with Desmodur T 100 in the Salmonella/Microsome Test

S9 mix	TA 1535	TA 100	TA 1537	TA 98
without	-ve	-ve	-ve	-ve
with	-ve	-ve	+ve	+ve

-ve = negative , w = weakly positive
+ve = positive, s = strongly positive

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene raised mutant counts well over negative control levels. This demonstrated the system's sensitivity and the activity of the S9 mix.

5.2 Tabulated Summary of Data

Summary of Mean Values without S9 Mix from Tables 1-8

Table and group µg/plate	Strain TA 1535	TA 100	TA 1537	TA 98
1-4				
0	13	54	8	21
8	12	55	9	15
40	11	55	8	14
200	11	40	5	8
1000	6	13	4	4
5000	-	-	-	-
Na-azide	746			
NF		247		
4-NPDA			52	54
5-8				
0	13	42	6	23
62.5	10	53	6	17
125.0	10	43	6	14
250.0	10	42	4	11
500.0	6	26	4	4
1000.0	3	12	2	3
2000.0	-	-	1	0
4000.0	-	-	-	-
Na-azide	553			
NF		195		
4-NPDA			49	69

Desmodur T 100
Salmonella/Microsome Test
Study No. T 5039111
BAYER AG

Summary of Mean Values with S9 Mix from Tables 1-12

Table and group µg/plate	Strain TA 1535	TA 100	TA 1537	TA 98
1-4 10% S9				
0	19	60	10	32
8	18	75	9	34
40	18	95	12	45
200	20	110	19	64
1000	12	43	20	55
5000	-	-	-	-
2-AA	229	746	272	815
5-8 30% S9				
0	22	68	12	30
62.5	22	74	14	31
125.0	19	85	14	42
250.0	25	98	14	39
500.0	18	55	19	60
1000.0	19	56	11	54
2000.0	-	-	-	-
4000.0	-	-	-	-
2-AA	105	305	113	268
9-12 10% S9				
0	33	101	19	35
62.5	34	85	31	40
125.0	36	82	32	39
250.0	40	82	36	48
500.0	47	74	27	52
1000.0	29	46	20	37
2000.0	-	-	-	-
4000.0	-	-	-	-
2-AA	201	493	201	552

Summary of Mean Values with S9 Mix from Tables 13-16

Table and group µg/plate	Strain TA 1535	TA 100	TA 1537	TA 98
12-14 30% S9				
0			9	29
50.0			12	41
100.0			12	52
200.0			10	49
400.0			13	64
600.0			21	106
800.0			21	86
1000.0			20	46
2-AA			65	623
15-16 10% S9				
0			10	34
50.0			13	49
100.0			15	64
200.0			15	84
400.0			36	138
600.0			41	106
800.0			36	124
1000.0			29	39
2-AA			266	1470

Summary of Mean Values with S9 Mix from Tables 17-20

Table and group µg/plate	Strain TA 1535	TA 100	TA 1537	TA 98
17-18 30% S9				
0			12	39
50.0			16	52
100.0			16	76
200.0			25	76
400.0			22	87
600.0			45	93
800.0			41	128
1000.0			42	58
2-AA			80	509
19-20 10% S9				
0			19	54
50.0			18	64
100.0			23	104
200.0			44	117
400.0			52	153
600.0			51	172
800.0			49	125
1000.0			41	117
2-AA			233	1245

6. Assessment

Doses of Desmodur T 100 up to 125 µg per plate did not induce any bacteriotoxic effect in the Salmonella/microsome test: Total bacteria counts remained unchanged and no growth inhibition was observed. The substance revealed weak, strain-specific bacteriotoxic effects at doses between 200 and 1000 µg per plate. The corresponding plates could be used for assessment purposes. Strong bacteriotoxic effects were observed for the dose of 2000 µg per plate and for higher doses which prohibited the assessment of the plates.

Substance precipitation occurred at the dose of 800 µg per plate and above.

Evaluation of individual dose groups, with respect to relevant assessment parameters (dose effect, reproducibility), revealed clear, biologically relevant variations from the respective negative controls for TA 98 and TA 1537. These were regarded as mutagenic effects of Desmodur T 100. Since the lowest effective doses at which this finding was reproducible, was in the bacteriotoxic dose range, the Salmonella/microsome test showed Desmodur T 100 to be a weak but definite mutagen under the conditions of the test.

In spite of the low doses used, positive controls increased the mutant counts significantly over negative control levels which demonstrated the system's high sensitivity.

Due to this sensitivity, evidence of mutagenic effects of Desmodur T 100 could be found at assessable doses up to 1000 µg per plate in Salmonella typhimurium TA 98 and TA 1537.

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8. Historical Controls

Summary of historical negative and positive controls of experiments performed from January to June 1988 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water -	14	2	97	9	8	1	17	2
DMSO -	13	2	94	15	8	1	17	2
DMF -	12	2	87	11	8	1	19	3
ethanol -	15	3	69	7	7	1	22	3
acetone -	10	2	85	10	7	1	18	2
EGDE[2] -	18		117		10		21	
Na-azide-	839	115						
NF -			382	46				
4-NPDA -					90	13	109	20
30%								
water +	14	3	134	10	8	2	29	3
DMSO +	15	3	124	14	9	2	29	3
DMF +	14	3	113	9	9	2	31	5
ethanol +	20	2	105	6	6	1	30	3
acetone +	14	1	134	25	11	2	34	3
EGDE[2] +	18		159		9		35	
2-AA +	282	63	601	164	66	17	532	160
10%								
water +	14	4	123	4	9		33	
DMSO +	14	2	111	13	8	1	33	5
DMF +	--		72		9		27	
ethanol +	23		87	6	8		38	
acetone +	13		85		7		29	
2-AA +	357	67	1422	428	298	65	1323	323

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from July to December 1988 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water	-	14	3	97	9	8	2	20	5
DMSO	-	14	2	93	25	8	1	19	10
DMF	-	12	2	70	4	7	1	13	1
ethanol	-	10	2	71	2	7	1	21	3
acetone	-	15	2	138	10	8	3	39	6
Na-azide	-	822	137						
NF	-			412	42				
4-NPDA	-					88	19	124	25
30%									
water	+	12	2	144	15	10	2	35	6
DMSO	+	16	2	124	15	10	2	32	5
DMF	+	14	3	117	14	9	2	31	6
ethanol	+	17	3	90	4	8	1	39	2
acetone	+	13	4	177	35	9	2	43	8
2-AA	+	261	69	755	196	93	21	583	171
10%									
DMSO	+	7	1	110	12	9	1	32	5
DMF	+	11		121		6		26	
2-AA	+	348	70	1544	572	416	75	1499	423

Summary of historical negative and positive controls of experiments performed from January to June 1989 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water -	10	3	91	11	7	1	18	3
DMSO -	9	3	84	16	7	2	16	2
DMF -	7	1	60	4	6	1	14	2
ethanol -	10	2	73	12	7	2	18	4
acetone -	9	-	100	--	7	-	18	-
EGDE[2] -	8	2	69	16	6	2	17	4
Na-azide-	721	110						
NF -			359	61				
4-NPDA -					75	13	119	35
30%								
water +	14	2	133	12	9	2	32	8
DMSO +	14	3	114	18	9	1	28	4
DMF +	14	2	100	9	8	2	25	4
ethanol +	17	3	118	12	10	2	37	8
acetone +	15	-	138	--	13	-	32	-
EGDE[2] +	14	2	115	25	11	2	27	8
2-AA +	195	33	633	127	63	28	392	133
10%								
DMSO +	12	2	105	28	7	2	25	4
DMF +	--	-	---	--	7	-	31	-
2-AA +	267	27	1455	348	283	64	1547	289

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from July to December 1989 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
DMSO -	10	4	72	6	7	4	16	5
DMF -	9	4	57	15	8	2	17	5
ethanol -	8	3	57	12	6	1	14	6
acetone -	15	-	96	--	6	-	13	-
EGDE[2] -	8	-	63	--	6	-	21	-
Na-azide -	853	147						
NF -			326	47				
4-NPDA -					91	26	87	25
30%								
DMSO +	14	2	89	7	11	2	23	2
DMF +	15	3	87	6	11	4	26	4
ethanol +	11	6	79	13	8	2	23	5
acetone +	21	-	96	--	11	-	20	-
EGDE[2] +	13	-	87	--	11	-	26	-
2-AA +	157	42	500	83	73	22	498	101
10%								
DMSO +	14	5	91	7	10	1	24	4
ethanol +	11	-	53	--	4	-	18	-
2-AA +	158	54	1464	152	289	117	1294	113

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from January to June 1990 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water -	15	3	74	10	7	1	22	5
DMSO -	12	2	72	13	8	2	17	3
DMF -	10	4	65	10	7	2	10	6
methanol-	17		87		7		19	
ethanol -	13	3	77	11	8	2	19	2
acetone -	10	1	69	4	6	1	11	2
EGDE[2] -	14	4	95	14	8	1	18	5
Na-azide-	799	108						
NF -			268	48				
4-NPDA -					52	12	81	14
30%								
water +	18	2	108	17	9	2	27	5
DMSO +	18	3	86	11	9	2	27	3
DMF +	13	3	97	17	7	3	20	5
methanol+	22		121		11		28	
ethanol +	19	3	98	15	8	2	29	4
acetone +	13	1	104	8	7	3	22	3
EGDE[2] +	15	2	97	9	9	3	28	8
2-AA +	161	39	509	130	48	15	379	54
10%								
DMSO +	18	2	89	20	11	4	30	6
ethanol +	16		85		8		29	
acetone +			107				17	
2-AA +	214	49	1196	181	235	38	1140	284

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from July to December 1990 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR
water -	13	2	105	16	9	1	21	4
DMSO -	14	2	105	7	8	1	21	3
DMF -	13	2	82	16	6	2	12	4
methanol-	13	1	105	16	8	1	21	4
ethanol -	12	3	93	14	9	1	22	3
acetone -	12	2	116	2	6	1	23	1
EGDE[2] -	13	2	112	15	8	2	18	3
Na-azide-	882	114						
NF -			380	60				
4-NPDA -					48	9	71	15
30%								
water +	18	3	143	15	11	2	29	3
DMSO +	17	2	137	5	10	2	28	4
DMF +	15	3	109	14	10	1	23	3
methanol+	22	2	144	16	11	2	33	3
ethanol +	19	3	118	18	10	1	39	7
acetone +	13	1	131	4	9	1	26	1
EGDE[2] +	18	3	135	14	11	2	32	5
2-AA +	175	41	800	243	84	17	485	93
10%								
DMSO +	16	2	127	19	9	3	32	5
acetone +	12		124		10		26	
EGDE[2] +			140					
2-AA +	179	69	1321	148	298	39	1206	168

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from January to June 1991 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water -	12	3	111	10	9	2	28	5
DMSO -	13	2	113	14	10	2	30	3
DMF -	9	-	80	--	7	-	23	-
methanol-	11	2	105	14	8	2	29	5
ethanol -	12	1	96	15	9	2	31	5
acetone -	10	-	55	--	5	-	21	-
EGDE[2] -	11	3	108	5	8	1	23	8
Na-azide-	623	102						
NF -			398	56				
4-NPDA -					49	10	89	20
30%								
water +	16	3	152	15	12	2	38	7
DMSO +	18	3	154	11	12	2	40	7
DMF +	11		84	--	9	-	29	-
methanol+	23	5	152	7	10	3	48	10
ethanol +	19	3	127	17	10	3	43	6
acetone +	14	-	84	--	14	-	18	-
EGDE[2] +	15	4	132	6	8	1	40	9
2-AA +	182	33	800	163	86	24	472	105
10%								
water +	15	-	102	--	5	-	46	-
DMSO +	16	3	132	5	10	1	39	4
methanol+	--	-	150	--	--	-	--	-
2-AA +	208	48	1408	216	314	14	754	369

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from July to December 1991 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water	-	12	3	89	10	9	3	27	4
buffer	-	13	2	97	10	8	1	25	2
DMSO	-	12	3	92	15	9	1	24	4
DMF	-	7		75		7		17	
methanol	-	10	1	84	11	8	1	25	3
ethanol	-	12	4	80	8	8	3	23	4
acetone	-	12	2	87	6	8	1	26	4
EGDE[2]	-	14	3	107	22	8	1	26	5
Na-azide	-	605	122						
NF	-			339	52				
4-NPDA	-					53	9	79	17
30%									
water	+	19	4	138	21	13	2	33	4
buffer	+	17		159		13		38	
DMSO	+	19	3	130	11	10	2	33	4
DMF	+	11		142		9		32	
methanol	+	25		134		12		37	
ethanol	+	18	5	119	19	11	2	37	2
acetone	+	18	2	111	9	13		28	11
EGDE[2]	+	22	4	144	11	13	3	32	3
2-AA	+	164	38	727	139	91	32	520	161
10%									
water	+	16	4	113	18	10	3	33	5
buffer	+	14		94		10		34	
DMSO	+	16	2	118	14	10	3	31	3
DMF	+	15		114	6	11		21	
methanol	+	16		111		9		29	
ethanol	+	19	3	94	6	12	2	32	2
acetone	+	17		112		11		32	
EGDE[2]	+	20	2	153	11	11	1	34	5
2-AA	+	197	50	1431	260	304	116	1097	207

[2]) Ethylene glycol dimethylether

9. Stability in Vehicle

Results of the analysis for stability of
Desmodur T 100
in the vehicle at room temperature

nominal value in mg/ml	content in % after storage time 0 hrs	4 hrs
0.08	118.8	118.8
50	105	103.8

According to these results Desmodur T 100 is stable in the vehicle at room temperature at concentrations ranging from 0.08 mg/ml to 50 mg/ml for at least four hours.

9

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 1

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 18, 1992
Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{-8}	QUOTIENT -S9	+S9
EGDE	12	13	3	18	19	4	282	30.8	1.0	1.0
	14			16			333			
	16			24						
	9			16						
8	13	12	3	24	18	7	277	27.6	0.9	1.0
	9			12			274			
	11			13						
	15			24						
40	12	11	3	12	18	6	276	27.9	0.9	1.0
	8			22			282			
	11			23						
	14			14						
200	12	11	2	17	20	3	289	28.7	0.8	1.1
	12			22			284			
	11			22						
	7			18						
1000	8 P	6	2	11 P	12	3	171 P	21.3**	0.5	0.6
	6 P			12 P			255 P			
	4 P			15 P						
	5 P			8 P						
5000	P	/	/	P	/	/	P	/	/	/
Na-azide 10	829	746	63	%	/	/	317	31.6	58.5*	/
	742						314			
	737									
	676									
2-AA 3	%	/	/	249	229	34	303	29.9	/	12.4*
				194			295			
				207						
				267						

*: Mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
**: Bacteriotoxic effect
P: Precipitation
SD: Standard-Deviation
+S9. with S9 Mix

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AMES TEST with : Desmodur T 100

Table : 2

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 18, 1992
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	45	54	8	58	60	4	100	9.8	1.0	1.0
	49			64			95			
	56			62						
	64			56						
8	57	55	11	71	75	14	102	11.7	1.0	1.2
	67			85			131			
	41			56						
	56			87						
40	58	55	4	95	95	10	122	10.6	1.0	1.6
	51			107			89			
	52			83						
	58			93						
200	31	40	9	139	110	30	114	11.8	0.7	1.8
	48			125			121			
	48			106						
	33			70						
1000	15 P	13	5	44 P	43	19	118 P	13.9	0.2	0.7
	9 P			30 P			159 P			
	9 P			69 P						
	18 P			29 P						
5000	P	/	/	P	/	/	P	/	/	/
NF 0.2	237	247	17	%	/	/	106	13.1	4.6*	/
	253						155			
	267									
	230									
2-AA 3	%	/	/	667	746	80	108	11.5	/	12.4*
				697			122			
				777						
				844						

*: Mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
P: Precipitation
SD: Standard-Deviation
+S9: with S9 Mix

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AMES TEST with : Desmodur T 100

Table : 3

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 18, 1992
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	7	8	1	11	10	2	139	13.8	1.0	1.0
	8			11			137			
	8			9						
	9			8						
8	9	9	2	8	9	1	152	14.8	1.1	0.9
	8			10			143			
	7			9						
	11			9						
40	8	8	1	13	12	3	136	15.0	1.0	1.2
	9			13			163			
	6			8						
	9			13						
200	6	5	1	14	19	5	150	14.5	0.7	2.0
	5			21			139			
	6			26						
	4			16						
1000	3 P	4	1	18 P	20	6	150 P	14.7	0.5	2.1
	4 P			29 P			143 P			
	4 P			18 P						
	4 P			16 P						
5000	P	/	/	P	/	/	P	/	/	/
4-NPDA	49	52	7	%	/	/	159	15.8	6.5*	/
10	46						156			
	51									
	61									
2-AA	%	/	/	254	272	58	135	14.4	/	27.9*
3				302			152			
				332						
				200						

*: Mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
P: Precipitation
SD: Standard-Deviation
+S9: with S9 Mix

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AMES TEST with : Desmodur T 100

Table : 4

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 18, 1992
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	25	21	5	29	32	5	116	11.6	1.0	1.0
	14			32			116			
	23			28						
	23			39						
8	16	15	2	30	34	7	170	18.0	0.7	1.0
	16			40			189			
	14			26						
	13			38						
40	19	14	3	41	45	3	146	16.5	0.7	1.4
	12			48			184			
	14			44						
	12			46						
200	12	8	3	57	64	7	196	19.3	0.4	2.0*
	9			63			190			
	6			74						
	5			60						
1000	5 P	4	1	71 P	55	11	222 P	23.0	0.2	1.7
	4 P			50 P			237 P			
	2 P			51 P						
	3 P			49 P						
5000	P	/	/	P	/	/	P	/	/	/
4-NPDA 0.5	51	54	14	%	/	/	211	20.6	2.5*	/
	40						200			
	49									
	74									
2-AA 3	%	/	/	854	815	35	172	19.2	/	25.5*
				828			212			
				771						
				807						

*: Mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
P: Precipitation
SD: Standard-Deviation
+S9: with S9 Mix

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 5

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 24, 1992
Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	30% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	11 13 17 10	13	3	23 19 22 23	22	2	353 309	33.1	1.0	1.0
62.5	8 11 10 11	10	1	21 23 25 20	22	2	340 359	35.0	0.8	1.0
125.0	9 10 11 11	10	1	23 19 18 16	19	3	352 310	33.1	0.8	0.9
250.0	9 12 11 9	10	2	25 24 28 23	25	2	348 290	31.9	0.8	1.1
500.0	5 6 5 7	6	1	24 11 14 21	18	6	382 296	33.9	0.5	0.8
1000.0	1 P 2 P 5 P 4 P	3	2	17 P 22 P 19 P 19 P	19	2	384 P 291 P	33.8	0.2	0.9
2000.0	P	/	/	P	/	/	P	/	/	/
4000.0	P	/	/	P	/	/	P	/	/	/
Na-azide 10	609 560 532 509	553	43	%	/	/	385 333	35.9	43.3*	/
2-AA 3	%	/	/	147 96 81 96	105	29	374 365	37.0	/	4.8*

*: Mutagenic effect
M: Mean
-S9: without S9 Mix
%: not tested
SD: Standard-Deviation
+S9: with S9 Mix
P: Precipitation

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 6

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 24, 1992
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	30% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	37 38 45 47	42	5	80 73 51 66	68	12	250 199	22.5	1.0	1.0
62.5	56 60 48 48	53	6	85 65 75 69	74	9	216 235	22.6	1.3	1.1
125.0	41 52 44 34	43	7	98 100 83 60	85	18	209 160	18.5	1.0	1.3
250.0	46 34 47 39	42	6	103 94 108 86	98	10	229 174	20.2	1.0	1.4
500.0	27 24 27 24	26	2	58 68 42 51	55	11	218 205	21.2	0.6	0.8
1000.0	18 P 7 P 6 P 16 P	12	6	55 P 65 P 54 P 49 P	56	7	163 P 185 P	17.4	0.3	0.8
2000.0	P	/	/	P	/	/	P	/	/	/
4000.0	P	/	/	P	/	/	P	/	/	/
NF 0.2	187 235 173 186	195	27	%	/	/	241 192	21.7	4.7*	/
2-AA 3	%	/	/	308 315 330 265	305	28	210 260	23.5	/	4.5*

*: Mutagenic effect
M: Mean
-S9: without S9 Mix

%: not tested
SD: Standard-Deviation
+S9: with S9 Mix

P: Precipitation

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 7

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 24, 1992
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	30% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	7 5 6 5	6	1	14 11 13 11	12	2	366 316	34.1	1.0	1.0
62.5	7 6 5 4	6	1	13 11 15 17	14	3	399 319	35.9	1.0	1.1
125.0	7 6 5 7	6	1	13 16 14 11	14	2	331 357	34.4	1.1	1.1
250.0	4 4 3 4	4	1	14 13 13 16	14	1	267 255	26.1	0.7	1.1
500.0	4 6 4 3	4	1	18 19 18 19	19	1	366 332	34.9	0.7	1.5
1000.0	1 P 2 P 1 P 2 P	2	1	28 P 29 P 41 P 25 P	31	7	317 P 335 P	32.6	0.3	2.5*
2000.0	0 P 0 P 2 P 1 P	1	1	P	/	/	P	/	0.1	/
4000.0	P	/	/	P	/	/	P	/	/	/
4-NPDA 10	51 52 53 41	49	6	%	/	/	337 345	34.1	8.6*	/
2-AA 3	%	/	/	119 105 130 97	113	15	306 310	30.8	/	9.2*

*: Mutagenic effect
M: Mean
-S9: without S9 Mix

%: not tested
SD: Standard-Deviation
+S9: with S9 Mix

P: Precipitation

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 8

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 24, 1992
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	30% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	25 18 28 19	23	5	30 30 33 27	30	2	168 203	18.6	1.0	1.0
62.5	19 15 18 16	17	2	40 30 27 28	31	6	256 203	23.0	0.8	1.0
125.0	16 18 12 10	14	4	43 34 40 50	42	7	235 205	22.0	0.6	1.4
250.0	14 10 11 7	11	3	45 39 36 36	39	4	291 271	28.1	0.5	1.3
500.0	5 2 3 4	4	1	58 63 63 57	60	3	269 274	27.2	0.2	2.0*
1000.0	5 P 0 P 3 P 3 P	3	2	49 P 54 P 69 P 43 P	54	11	212 214	21.3	0.1	1.8
2000.0	0 P 0 P 1 P 0 P	0	1	P	/	/	161 161	16.1	<0.1	/
4000.0	P	/	/	P	/	/	220 197	20.9	/	/
4-NPDA 0.5	48 74 80 72	69	14	%	/	/	291 326	30.9	3.0*	/
2-AA 3	%	/	/	299 260 251 261	268	21	212 222	21.7	/	8.9*

*: Mutagenic effect
M: Mean
-S9: without S9 Mix

%: not tested
SD: Standard-Deviation
+S9: with S9 Mix

P: Precipitation

B A Y E R A.G.
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WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 9

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 24, 1992
Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT +S9
EGDE	29 39 33 31	33	4	349 370	36.0	1.0
62.5	32 35 31 36	34	2	374 435	40.5	1.0
125.0	42 C 29 38	36	7	266 230	24.8	1.1
250.0	35 38 39 47	40	5	250 209	23.0	1.2
500.0	41 48 46 53	47	5	217 268	24.3	1.4
1000.0	31 P 21 P 27 P 37 P	29	7	258 P 222 P	24.0	0.9
2000.0	P	/	/	P	/	/
4000.0	P	/	/	P	/	/
2-AA 3	186 189 194 236	201	23	363 350	35.7	6.1*

*: Mutagenic effect
M: Mean
P: Precipitation
+S9: with S9 Mix
C: Contamination
SD:Standard-Devitation

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 10

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 24, 1992
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT +S9
EtOE	95 120 107 81	101	17	228 252	24.0	1.0
62.5	99 81 74 84	85	11	263 278	27.1	0.8
125.0	60 93 91 85	82	15	260 287	27.4	0.8
250.0	82 96 67 81	82	12	274 250	26.2	0.8
500.0	61 67 85 84	74	12	248 238	24.3	0.7
1000.0	55 P 37 P 53 P 38 P	46	10	227 P 245 P	23.6	0.5
2000.0	P	/	/	P	/	/
4000.0	P	/	/	P	/	/
2-AA 3	478 500 504 489	493	12	298 203	25.1	4.9*

*: Mutagenic effect
SD: Standard-Devitation
P: Precipitation
+S9: with S9 Mix
M: Mean

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 11

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept. 24, 1992
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT +S9
EGDE	18 20 19 20	19	1	306 266	28.6	1.0
62.5	32 26 28 36	31	4	278 260	26.9	1.6
125.0	29 28 36 35	32	4	209 124	16.7	1.7
250.0	36 30 35 41	36	5	263 246	25.5	1.8
500.0	27 25 28 C	27	2	199 205	20.2	1.4
1000.0	20 P 17 P 18 P 25 P	20	4	265 P 262 P	26.4	1.0
2000.0	P	/	/	P	/	/
4000.0	P	/	/	P	/	/
2-AA 3	197 221 183 204	201	16	345 329	33.7	10.5*

*: Mutagenic effect
M: Mean
P: Precipitation
+S9: with S9 Mix
C: Contamination
SD: Standard-Devitation

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AMES TEST with : Desmodur T 100

Table : 12

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Sept 24, 1992
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT +S9
EGDE	38 30 36 35	35	3	370 364	36.7	1.0
62.5	45 34 38 42	40	5	260 309	28.5	1.1
125.0	40 37 44 36	39	4	274 230	25.2	1.1
250.0	35 46 56 55	43	10	289 249	26.9	1.4
500.0	51 53 56 46	52	4	258 259	25.9	1.5
1000.0	32 P 30 P 38 P 46 P	37	7	255 P 288 P	27.2	1.1
2000.0	P	/	/	P	/	/
4000.0	P	/	/	P	/	/
2-AA 3	597 560 543 508	552	37	308 296	30.2	15.9*

*: Mutagenic effect
SD: Standard-Deviation
P: Precipitation
+S9: with S9 Mix
M: Mean

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 13

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Diver
Date : Oct. 8, 1992
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 30% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT +S9
EGDE	7 7 9 11	9	2	226 212	21.9	1.0
50	10 8 14 14	12	3	224 209	21.7	1.4
100	13 12 14 9	12	2	225 179	20.2	1.4
200	13 10 10 7	10	2	170 194	18.2	1.2
400	12 10 14 17	13	3	240 210	22.5	1.6
600	25 22 21 17	21	3	244 225	23.5	2.5*
800	24 20 19 C	21	3	198 208	20.3	2.5*
1000	20 P 20 P 18 P 20 P	20	1	226 199	21.3	2.3*
2-AA 3	71 68 55 66	65	7	209 222	21.6	7.6*

*: Mutagenic effect
M Mean
P: Precipitation
+S9: with S9 Mix
C: Contamination
SD: Standard-Devitation

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 14

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Oct. 8, 1992
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 30% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT +S9
EGDE	30 30 26 26	29	2	62 51	5.7	1.0
50	51 39 35 39	41	7	154 127	14.1	1.4
100	51 48 53 55	52	3	168 128	14.8	1.8
200	50 50 45 51	49	3	144 133	13.9	1.7
400	59 62 62 73	64	6	161 143	15.2	2.2*
600	124 103 96 101	106	12	168 143	15.6	3.7*
800	63 60 116 106	86	29	150 146	14.8	3.0*
1000	45 F 52 P 49 P 37 P	46	7	165 159	16.2	1.6
2-AA 3	637 633 645 575	623	32	175 204	19.0	21.7*

*: Mutagenic effect
SD: Standard-Deviation
P: Precipitation
+S9: with S9 Mix
M: Mean

B A Y E R A.G.
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WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 15

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Oct. 8, 1992
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT +S9
EGDE	9	10	1	213	20.9	1.0
	11			204		
	10					
	11					
50	12	13	2	227	20.8	1.2
	14			189		
	10					
	15					
100	15	15	2	207	22.6	1.5
	17			244		
	15					
	13					
200	16	15	1	249	21.9	1.5
	15			189		
	16					
	14					
400	50	36	9	207	18.9	3.5*
	30			170		
	31					
	33					
600	44	41	8	202	21.2	4.0*
	51			221		
	35					
	33					
800	25 B	36	10	193	19.1	3.5*
	44 B			189		
	44 P					
	30 P					
1000	29 P	29	3	201	17.7	2.9*
	32 P			153		
	25 P					
	31 P					
2-AA 3	299	266	27	177	18.3	26.0*
	249			189		
	278					
	239					

*: Mutagenic effect
M: Mean
B: Background lawn reduced
+S9: with S9 Mix
P: Precipitation
SD: Standard-Devitation

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WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 16

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Oct. 8, 1992
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 10% +S9	M	SD	TITER Dilution per ml 10^{-6}	10^{-8}	QUOTIENT +S9
EGDE	35 36 34 30	34	3	105 99	10.2	1.0
50	46 50 57 44	49	6	196 161	17.9	1.5
100	57 68 60 70	64	6	185 177	18.1	1.9
200	102 84 67 83	84	14	161 200	18.1	2.5*
400	148 142 135 127	138	9	172 176	17.4	4.1*
600	99 117 119 88	106	15	165 178	17.2	3.1*
800	130 103 108 153	124	23	191 153	17.2	3.7*
1000	61 B 14 B 16 P 64 P	39	27	157 148	15.3	1.1
2-AA 3	1365 1669 1434 1413	1470	136	173 184	17.9	43.6*

*: Mutagenic effect
M: Mean
B: Background lawn reduced
+S9: with S9 Mix
P: Precipitation
SD: Standard-Devitation

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DEPARTMENT OF TOXICOLOGY
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WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 17

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Nov. 9, 1992
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	30% +S9	M	SD	Dilution per ml 10^{-6}	10^{-8}	+S9
EGDE	11	12	3	173	17.3	1.0
	9			172		
	13					
	16					
50	14	16	2	264	26.8	1.3
	19			271		
	16					
	16					
100	14	16	4	256	25.0	1.3
	19			243		
	12					
	20					
200	23	25	3	252	23.5	2.0
	30			218		
	23					
	24					
400	25 B	22	5	213	21.3	1.8
	15 B			212		
	22 B					
	25 B					
600	47 B	45	17	207	19.6	3.6*
	67 B			185		
	31 B					
	33 B					
800	33 B	41	8	166	16.0	3.3*
	48 B			153		
	48 P					
	35 P					
1000	54 B	42	9	92	13.2	3.4*
	41 B			171		
	40 P					
	33 P					
2-AA	82	80	11	274	24.2	6.5*
3	91			210		
	81					
	65					

*: Mutagenic effect
M: Mean
+S9: with (30%) S9-mix
P: Precipitation
SD: Standard-Deviation
B: Background lawn reduced

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AMES TEST with : Desmodur T 100

Table : 18

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Nov. 9,1992
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 30% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT +S9
EGDE	40 41 45 31	39	6	106 86	9.6	1.0
50	68 51 48 39	52	12	191 245	21.8	1.3
100	56 81 95 73	76	16	210 233	22.2	1.9
200	90 80 69 64	76	12	237 257	24.7	1.9
400	96 B 90 B 104 B 59 B	87	20	245 225	23.5	2.2*
600	116 B 100 B 80 B 77 B	93	18	266 261	26.4	2.4*
800	133 B 146 B 107 P 126 P	128	16	262 288	27.5	3.3*
1000	53 B 27 B 65 P 85 P	58	24	259 277	26.8	1.5
2-AA 3	513 545 504 474	509	29	250 219	23.5	13.0*

*: Mutagenic effect
M: Mean
+S9: with (30%) S9 mix
P: Precipitation
SD: Standard-Deviation
B: Background lawn reduced

B A Y E R A.G.
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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 100

Table : 19

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Nov. 9, 1992
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 10% +S9		M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT +S9
EGDE	18		19	3	250	23.4	1.0
	15				217		
	21						
	22						
50	24		18	4	254	25.9	0.9
	16				264		
	16						
	14						
100	24		23	3	220	23.1	1.2
	20				241		
	23						
	26						
200	55	B	44	11	276	26.9	2.3*
	35	B			262		
	51	B					
	35	B					
400	50	B	52	5	221	22.3	2.7*
	59	B			225		
	48	B					
	49	B					
600	49	B	51	7	228	21.8	2.7*
	57	B			208		
	42	B					
	56	B					
800	50	B	49	9	173	17.7	2.6*
	60	B			181		
	40	P					
	44	P					
1000	40	B	41	16	164	15.4	2.2*
	26	B			144		
	63	P					
	36	P					
2-AA	256		233	22	220	23.0	12.2*
3	220				240		
	209						
	245						

*: Mutagenic effect
M: Mean
+S9: with (30%) S9-mix
P: Precipitation
SD: Standard-Deviation
B: Background lawn reduced

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PHARMA RESEARCH CENTER
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AMES TEST with : Desmodur T 100

Table : 20

Study Number : T 5039111
Study Director : Dr.Gahlmann
Technician : Düver
Date : Nov. 9, 1992
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 10% +S9	M	SD	TITER Dilution per 10^{-6}	10^{-8}	QUOTIENT +S9
EGDE	49 49 59 59	54	6	220 226	22.3	1.0
50	64 67 62 63	64	2	284 278	28.1	1.2
100	85 106 92 131	104	20	319 276	29.8	1.9*
200	132 130 116 90	117	19	282 273	27.8	2.2*
400	168 B 164 B 130 B 150 B	153	17	264 267	26.6	2.8*
600	164 B 174 B 175 B 173 B	172	5	260 295	27.8	3.2*
800	149 B 159 B 62 P 128 P	125	44	243 262	25.3	2.3*
1000	109 B 136 B 140 P 84 P	117	26	264 257	26.1	2.2*
2-AA 3	1259 1232 1027 1461	1245	178	279 276	27.8	23.1*

*: Mutagenic effect
M: Mean
+S9: with (30%) S9-mix
P: Precipitation
SD: Standard-Deviation
B: Background lawn reduced

CERTIFICATE OF AUTHENTICITY

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Data produced 8 4 94 Marcia Tubolino
(Month) (Day) (Year) Camera Operator

Place Syracuse New York
(City) (State)

AMTEK
corp